APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
03/06/2006	
3. DATE RECEIVED BY STATE	**State Application Identifier**

1. * TYPE OF SUBMISSION

❍ Pre-application ● Application
❍ Changed/Corrected Application

4. Federal Identifier

5. APPLICANT INFORMATION *** Organizational DUNS:**6162000090000

* Legal Name:Elitra Pharmaceuticals, Inc.
Department: Division:
* Street1:3510 Dunhill St Street2:
* City:San Diego County: * State:CA * ZIP Code:92121
* Country: USA

Person to be contacted on matters involving this application

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Mr.	Harry	F.	Hixson	

Phone Number: 858-410-3030 Fax Number: 858-410-3040 Email: hhixson@elitra.com

6. * EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)*:
444444444

7. * TYPE OF APPLICANT
O: Small Business

8. * TYPE OF APPLICATION: ● New
❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

Other (Specify):

Small Business Organization Type
❍ Women Owned ❍ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)*:

9. * NAME OF FEDERAL AGENCY:
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

* Is this application being submitted to other agencies? ❍ Yes ● No
What other Agencies?

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Dominant Expression for New Targets in Candida Albicans

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*
N/A

13. PROPOSED PROJECT:

* Start Date	* Ending Date
07/01/2000	01/01/2002

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant	b. * Project
46	46

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Dr.	John	D.	Trawick	

Position/Title: Senior Research Scientist * Organization Name: Elitra Pharmaceuticals, Inc.
Department: Division:
* Street1: 3510 Dunhill St Street2:
* City: San Diego County: * State: CA * ZIP Code: 92121
* Country: USA
* Phone Number: 858-410-3030 Fax Number: 858-410-3040 * Email: jtrawick@elitra.com

16. ESTIMATED PROJECT FUNDING	
a. * Total Estimated Project Funding	$100,000.00
b. * Total Federal & Non-Federal Funds	$100,000.00
c. * Estimated Program Income	$0.00

17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● * I agree

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix
	Harry	A.	Hixson	

* Position/Title: President and CEO * Organization Name: Elitra Pharmaceuticals, Inc.

Department: Division:

* Street1: 3510 Dunhill St Street2:

* City: San Diego County: * State:CA * ZIP Code:92121

* Country: USA

* Phone Number: 858-410-3030 Fax Number: 858-410-3040 * Email: hhixson@elitra.com

*** Signature of Authorized Representative**	*** Date Signed**
Harry A. Hixson	09/01/1999

20. Pre-application File Name: Mime Type:

424 R&R and PHS-398 Specific
Table Of Contents

RESEARCH & RELATED Project/Performance Site Location(s)

Project/Performance Site Primary Location

Organization Name: Elitra Pharmaceuticals, Inc.

* Street1: 3510 Dunhill Street Street2:

* City: San Diego County: * State: CA * Zip Code: 92121 * Country: USA

File Name	Mime Type

Additional Location(s)

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved?	❍ Yes	● No
1.a. If YES to Human Subjects		
Is the IRB review Pending?	❍ Yes	❍ No
IRB Approval Date:		
Exemption Number:	_ 1 _ 2 _ 3 _ 4 _ 5 _ 6	
Human Subject Assurance Number		
2. * Are Vertebrate Animals Used?	❍ Yes	● No
2.a. If YES to Vertebrate Animals		
Is the IACUC review Pending?	❍ Yes	❍ No
IACUC Approval Date:		
Animal Welfare Assurance Number		
3. * Is proprietary/privileged information included in the application?	● Yes	❍ No
4.a. * Does this project have an actual or potential impact on the environment?	❍ Yes	● No
4.b. If yes, please explain:		
4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed?	❍ Yes	❍ No
4.d. If yes, please explain:		
5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators?	❍ Yes	● No
5.b. If yes, identify countries:		
5.c. Optional Explanation:		

6. * Project Summary/Abstract	0054-RR06 Summaryv01.pdf	Mime Type: application/pdf
7. * Project Narrative	3014-RR07 Narrativev01.pdf	Mime Type: application/pdf
8. Bibliography & References Cited	3746-RR08 BibReferencesv01.pdf	Mime Type: application/pdf
9. Facilities & Other Resources	2283-RR09 Facilities.pdf	Mime Type: application/pdf
10. Equipment	547-RR10 Equipment.pdf	Mime Type: application/pdf

Project Summary/Abstract

A screen for dominant negative genes will be used to identify targets and pathways in *Candida albicans*. The genes and pathways identified will be developed as new antifungal targets. An expression vector system suitable for screening libraries in *C. albicans* has been devised. Phase I of this project includes final construction and optimization of the expression vector and construction of cDNA libraries capable of identifying dominant negative mutants. Preliminary screening will begin in Phase I. Phase II of the project will entail identifying essential genes and processes by dominant negative mutagenesis and to develop screens for new antifungals based on these essential genes. The method proposed for identifying essential genes is ideal for *C. albicans*, a diploid human pathogen not normally amenable to genetic analysis, and can be automated.

Narrative

This project seeks to develop new antimicrobial agents suitable for treating fungal infections by identifying drug targets and pathways in the fungus *Candida albicans*.

Literature Cited

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Facilities

(to be used for the conduct of the proposed research).

Laboratory:

Elitra Pharmaceuticals' laboratories are located in La Jolla, CA, where many biotech companies, University of California, San Diego, the Salk Institute, and Scripps Research Institute are also located. The Elitra facilities occupy 11,900 square feet. The laboratories contain all necessary standard equipment standard for molecular biology including incubators, cold room, warm room, centrifuges, freezers, chemical hood, a biosafety containment level 2 facility, electroporators, phase contrast microscope, spectrophotometer, autoclaves, 5 PCR machines, ice machine, ABI 377 and 3700 DNA sequencers, chemical balances, etc. Also included is equipment for automated picking and replica gridding of bacterial and fungal colonies. We have added an automated chemical screening system that was in place in October of 1999.

Computer:

Informatics support includes an internal TCP/IP network connected to the Internet through a high-bandwidth connection, and both multi-processor Windows NT and Sun Microsystems' Solaris computers serving as computer and file servers.

Office:

Other:

Major Equipment

ABI 377 and DNA Sequencer

The ABI PRISM® 377 DNA Sequencer is a high throughput, high sensitivity device for analyzing DNA molecules labeled with multiple fluorescent dyes. The laser detection system and computerized display provide real-time visualization of the electrophoretic separation; the final data output is available in a variety of data formats.

ABI 3700 DNA Sequencers

Capillary electrophoresis system capable of sequencing up to 96 DNA templates per run; the manufacture states that this device is the ideal platform for sequencing PCR products less than 550 base pairs in length (http://www.gmi-inc.com/BioTechLab/ABI%20377.htm).

Colony pickers

Two of these will be available for this project and are capable of picking bacterial colonies at a rate of 1800 per hour.

Gridders

Two available, each can move bacterial cells from microtiter plates containing liquis or solid media into liquid or solid media.

Biomek Robots

These lab automation devices carry out a variety of tasks including serial dilutions, plasmid minipreps, set-up for PCR sequencing reactions, and PCR product cleanup.

RESEARCH & RELATED Senior/Key Person Profile

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
	John	D.	Trawick	

Position/Title: Senior Research Scientist
Department:

Organization Name: Elitra Pharmaceuticals, Inc.
Division:

* Street1: 3510 Dunhill St.
Street2:

* City:San Diego County: * State:CA * Zip Code:92121 * Country: USA

*Phone Number	Fax Number	* E-Mail
858-410-3030	858-410-3040	jtrawick@elitra.com

Credential, e.g., agency login: columbumpi

*** Project Role:** PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	8205-RSKPersonBiosketchTrawick.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person 1

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	John	Douglas	Trawick	PhD

Position/Title: Senior Research Scientist
Department:

Organization Name: Elitra Pharmaceuticals
Division:

* Street1: 1212 Main St
Street2:

* City: San Diego County: * State: CA * Zip Code: 92121 * Country: USA

*Phone Number	Fax Number	* E-Mail
(858) 555-1212		JDT@biz.com

Credential, e.g., agency login:

*** Project Role:** Co-PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	7922-RSKPersonBiosketchTrawick.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person 2

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	J.	Gordon	Foulkes	PhD

Position/Title: Executive Vice President
Department: Research and Development
Organization Name: Elitra Pharm
Division:
* Street1: 1212 Main Street
Street2:
* City: San Diego
County:
* State: CA
* Zip Code: 11111
* Country: USA

*Phone Number	Fax Number	* E-Mail
(858) 555-1212		JGF@biz.com

Credential, e.g., agency login:

*** Project Role:** Co-PD/PI
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	1394-RSKPersonBiosketchFoulkes.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person 3

Prefix	* First Name	Middle Name	* Last Name	Suffix
Dr.	William	A.	Fonzi	PhD

Position/Title: Associate Professor
Department:
Organization Name: Georgetown University
Division:
* Street1: 1212 Main Street
Street2:
* City: Washington
County:
* State: DC
* Zip Code: 11111
* Country: USA

*Phone Number	Fax Number	* E-Mail
(301) 555-1212		WAF@univ.edu

Credential, e.g., agency login:

*** Project Role:** Faculty
Other Project Role Category:

	File Name	Mime Type
***Attach Biographical Sketch**	8839-RSKPersonBiosketchFonzi.pdf	application/pdf
Attach Current & Pending Support		

	File Name	Mime Type
ADDITIONAL SENIOR/KEY PERSON PROFILE(S)		
Additional Biographical Sketch(es) (Senior/Key Person)		
Additional Current and Pending Support(s)		

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
John Douglas Trawick	Senior Research Scientist, Elitra Pharmaceuticals
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Gustavus Adolphus Coll., St.Peter, MN	B.A.	1976	Biology
Northern Illinois University, Dekalb, IL	M.S.	1979	Biological Sciences
University of Minnesota, Minneapolis, MN	Ph.D.	1984	Microbiology

A. Positions and Honors

1984-1985 Postdoctoral Fellow, Mayo Foundation, Rochester, MN. Research in actin gene expression in mammalian cells.

1985-1990 Postdoctoral Research Associate, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO. Expression and regulation of cytochrome oxidase genes in the yeast, Saccharomyces cerevisiae, nuclear-mitochondrial interactions in S. cerevisiae.

1990-1992 Assistant Professor, Department of Medicine, University of Colorado Health Sciences Center, Denver, CO. Research into the molecular biology and genetics of bile acid synthesis in rat hepatoma cells and assembly of lipoproteins.

1992-1996 Adjunct Assistant Professor, Department of Biology, San Diego State University. Research into the molecular biology and genetics of bile acid synthesis in rat hepatoma cells and assembly of lipoproteins.

1992-present Adjunct Assistant Professor, Department of Biology, San Diego State University.

1996-1997 Course instructor, Biology Departments San Diego State University and University of San Diego.

1997-present Senior Research Scientist, Drug Development Dept., Elitra Pharmaceuticals. Target evaluation and validation in E. coli and Staphylococcus aureus. Target discovery in Salmonella typhimurium, target discovery in Candida *albicans*, vector development and improvement, and new organism evaluation.

Patent applications

Genes identified as required for proliferation in Escherichia coli. 2000 Inventors: Zyskind, J. W., Ohlsen, K.L., **Trawick, J.D.,** Forsyth, R. A., Froelich, J. M., Carr, G. J., Yamamoto, R. T., Xu, H. **WO 00/44906**
Identification of essential genes in prokaryotes. 2001. Haselbeck, R., Ohlsen, K. L., Zyskind, J. W., Wall, D., **Trawick, J. D**., Carr, G. J., Yamamoto, R.T., Xu, H. H. **WO 01/70955**

B. Selected Peer-Reviewed Publications (in chronological order)

Kline, B., Seelke, R. and **Trawick, J.** Replication and incompatibility functions in mini-F plasmids. *In* Levy, S.B., Clowes, R.L., and Koenig, E.L., eds., Molecular biology, pathogenicity, and ecology of bacterial plasmids, pp. 317-326. Proceedings of the International Plasmid Conference on Molecular Biology, Pathogenicity, and Ecology of Bacterial Plasmids, January 5-9, 1981, Santo Domingo, Dominican Republic, Plenum Press, NY.
Kline, B.C., Seelke, R.W., **Trawick, J.D.,** Levy, S.B. and Hogan, J. Genetic studies on the maintenance of mini-F plasmids. *In* Proceedings of the Third Tokyo Symposium on Mechanisms of Antibiotic Resistance, October, 1981, Tokyo, Japan 1984.
Seelke, R.W., Kline, B.C., **Trawick, J.D.,** and Ritts, G.D. 1982. Genetic studies of F plasmid maintenance genes involved in copy number control, incompatibility and partitioning. *Plasmid* **7:** 163-179.
Kline, B.C. and **Trawick, J**. 1983. Identification and characterization of a second copy number control gene in mini-F plasmids. *Molec. Gen. Genet.* **192:** 408-415.

Trawick, J.D. and Kline, B.C. 1985. A two-stage molecular model for control of mini-F replication. *Plasmid* **13:** 59-69.
Wright, R.M., **Trawick, J.D.,** Trueblood, C.E., Patterson, T.E., and Poyton, R.O. Organization and expression of nuclear genes for yeast cytochrome c oxidase. *In*: Cytochrome systems: Molecular biology and bioenergetics, pp. 49-56. ed. S. Papa. 1987. Plenum Press, NY.
Trawick, J.D., Wright, R.M., and Poyton, R.O. 1989. Transcription of yeast COX6, the gene for subunit VI of the cytochrome coxidase of S. cerevisiae, is dependent on heme and on the HAP2 gene. *J. Biol. Chem.* **264:** 7005-7008.
Trawick, J.D., Rogness, C.R., and Poyton, R.O. 1989. Identification of an upstream activation site and other cis-acting elements required for transcription of COX6 from Saccharomyces cerevisiae. *Mol. Cell. Biol.* **9:** 5350-5358.
Farrell, L.E., **Trawick, J.D.,** and Poyton, R.O. Mitochondrial-nuclear interactions: transcription of nuclear COX genes in yeast is reduced in cells that lack a mitochondrial genome. *In*: Structure, function, and biogenesis of energy transfer systems, pp.131-134, ed. E. Quaglieriello, S. Papa, F. Palmieri, and C. Saccone. 1990. Elsevier Press.
Trawick, J.D., Simon, F.R., Kraut, N.,and Poyton, R.O. 1992. Regulation of Yeast COX6 by the General Transcription Factor ABF1 and Separate HAP2 and Heme Responsive Elements. *Mol. Cell. Biol.* **12:** 2301-2314.
Leighton, J.K., Dueland, S., Straka, M.S., **Trawick, J.,** and Davis, R.A. 1991. Activation of the silent endogenous cholesterol-7-alphahydroxylase gene in rat hepatoma cells: A new complementation group having resistance to 25-hydroxycholesterol. *Mol. Cell. Biol.* **11:** 2049-2056.
Davis, R.A., Dueland, S. and **Trawick, J.** 1992. Bile Acid Synthesis and the Enterohepatic Circulation: Processes Regulating Total Body Cholesterol Homeostasis. *In* Molecular Genetics of Coronary Heart Disease and Stroke. Lusis, A., Rotter, J. and Sparkes, R.S., eds. Karger Press.
Thrift, R., Drisko, J. Dueland, S., **Trawick, J.D**., and Davis, R.A.,. 1992. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in a nonhepatic cell line.*Proc. Natl. Acad. Sci. USA.* **89:**9161-9165.
Dueland, S., **Trawick, J.D.,** Nenseter, M.S., MacPhee, A.A., and Davis, R.A. 1992. Expression of 7alpha-hydroxylase in non-hepatic cells results in liver phenotypic resistance of the low density lipoprotein receptor to cholesterol repression. *J. Biol. Chem.* **267:** 22695-22698.
Trawick, J. D.,Lewis, K.D., Moore, G.L., Simon, F.R., and Davis, R.A. 1996. Rat hepatoma L35 cells, a liver-differentiated cell line, display resistance to bile acid repression of cholesterol 7 alpha-hydroxylase. *J. Lipid Res.* **37:** 588-599.
Moore, G. L., Drevon, C. A., Machleder, D., Lusis, A. J., **Trawick, J. D**., Unson, M. A., McClelland, A., Roy, S., Lyons, R., Jambou, R., and Davis, R.A. 1997. Expression of human cholesterol 7 alpha-hydroxylase in atherosclerosis-susceptible mice via adenovirus infection. *Biochem. J.* **324:** 863-867.
Dueland, S., France, D., Wang, S.-L., **Trawick, J. D.,** and R. A. Davis. 1997. Cholesterol-7alpha-hydroxylase influences the expression of hepatic Apo AI in two inbred mouse strains displaying different susceptibilities to atherosclerosis and in hepatoma cells. *J. Lipid Res*. **38:** 1445-1453.
Trawick, J. D., Shui-Long Wang, David Bell, and R.A. Davis.1997. Transcriptional induction of 7 alpha-hydroxylase by dexamethasone in L35 hepatoma cells requires sulfhydryl reducing agents. *J. Biol. Chem*. **272:** 3099-3102.
R. A. Forsyth, R. J. Haselbeck, K. L. Ohlsen, R. T. Yamamoto, H. Xu, **J. D. Trawick**, D. Wall,L. Wang, V. Brown-Driver, J. M. Froelich, Kedar G. C., P. King, M. McCarthy, C. Malone, B. Misiner, D. Robbins, Z Tan, Z.-y. Zhu, G. Carr, D. A. Mosca, C. Zamudio, J. G. Foulkes & J. W. Zyskind. 2002. A genome-wide strategy for the identification of essential genes in *Staphylococcus aureus Molec. Microbiol*. **43 (6):** 1387-1400.

C. Research Support

Task Force Leader of a task force in Elitra collaboration with Merck. 2001-present. Organize and carry out efforts to build proprietary Elitra genetic system for identification of cellular targets “hit” by active compounds in the human pathogen, *S. aureus*. Co-leader of task force, designed principle genetic tools in effort and have overseen efforts of several person team in implementing these genetic tools.
Task Force leader of target validation for *E.coli* and *S. aureus* genes. 2000-present. Organized and executed efforts to validate essential gene targets recognized in Elitra genetic screening. Responsible for Elitra validation of essential gene targets in both of these organisms.

Member of team, Collaboration between Elitra and LG Chem. 2000-present. Responsible for functional evaluation of targets presented to LG Chem (Republic of Korea) as part of Elitra collaboration. Has evolved into responsibility for functional (i.e., biological role) of potential antibacterial targets in Elitra collaborations with other pharmaceutical firms.
Leader of team for essential gene identification in *Salmonella enterica* Typhimurium. 1999. Led effort to apply Elitra genetic technology to *Salmonella enterica*. Led effort to improve genomic library construction and genetic screening.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
J. Gordon Foulkes	Executive Vice President, Research and Development
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Dundee, Scotland	Ph.D.	1979	Biochemistry
University College Cardiff, Wales	B.Sc.	1976	Biochemistry

A. Positions and Honors

1980–1981 Postdoctoral fellow, University of Colorado, Denver, Colorado
1982–1984 Senior fellow, Massachusetts Institute of Technology, Massachusetts
1984–1987 Tenured Member of the Scientific Staff, The Medical Research Council (MRC), National Institute for Medical Research, London, U.K
1987–1990 Director of Therapeutics, Oncogene Science, Inc., New York, NY
1990–1992 Vice President and Director of Therapeutics, Oncogene Science, Inc., New York, NY
1992–1995 Vice President and Chief Scientific Officer, Oncogene Science, Inc., New York, NY
1994–1996 Appointed to the Office of the Chief Executive and the Board of Directors, Oncogene Science, Inc., New York, NY
1996–1998 Chief Technical Officer and Member of the Board of Directors, Aurora Biosciences Corporation, San Diego, CA
1999–Present Executive Vice President, Research and Development, Elitra Pharmaceuticals, San Diego, CA

B. Selected Peer-Reviewed Publications (in chronological order)

Over 50 major publications and reviews prior to joining Oncogene Science in 1987. Examples:
Discovery and characterization of mammalian protein-tyrosine phosphatases: *J. Biol. Chem. 258*, 431-438; FEBS Lett. 130, 197-200.
Discovery in transformed cells of tyrosine phosphorylated nuclear proteins: *Nature 325*, 552-554.
Development of the first bacterial expression system for purification of a tyrosine kinase: *J. Biol. Chem. 260*, 8070-8077.
Identification of serine/tyrosine protein kinase cascade systems. *Proc. Natl. Acad. Sci. U.S.A. 82*, 272-276; EMBO J. 4, 3173-3178; *Proc. Natl. Acad. Sci. U.S.A. 84*, 4408-4412.
Identification of protein phosphatases in translational control. *Proc. Natl. Acad. Sci. U.S.A. 82*, 272-276; *Proc. Natl. Acad. Sci. U.S.A. 79*, 7091-7096; *J. Biol. Chem. 258*, 1439-1443.
Discovery of a new human oncogene. *Nature 325*, 635-637.
Cloning of TGF-β3. *Proc. Natl. Acad. Sci. USA 85*, 4715-4719.

C. Research Support

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
William A. Fonzi	Associate Professor
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Texas A&M University, College Station, TX	B.S.	1975	Zoology
Texas A&M University, College Station, TX	Ph.D.	1981	Microbiology
University of California, Irvine, CA	Post-Doc	1981-1985	Molecular Genetics

A. Positions and Honors

Professional Positions:

1998–Present Associate Professor Georgetown University
10/94–1998 Assistant Professor Georgetown University
7/94–10/94 Associate Adjunct Prof. UC Irvine
9/89–6/94 Asst. Adjunct Prof. UC Irvine
9/85–9/89 Res. Associate UC Irvine

Awards and Other Professional Activities:

1997 Burroughs Wellcome Scholar of Molecular PathogenicMycology
1998 to present Editorial Board, Revista IberoAmericana de Micologia
1999 to present Editorial Board, Infection and Immunity
2000-2002 Editorial Board, Journal of Bacteriology
1999 external reviewer, International Institute of Genetics and Biophysics, Naples, Italy
2000 ad hoc reviewer, NIH Bacteriology Mycology II
2000 ad hoc reviewer, Research Grants Council of Hong Kong
2000 ad hoc reviewer, North Carolina Biotechnology Center

Research Projects Ongoing or Completed During the Last 3 Years:

"Regulation of Dimorphism in Candida albicans"
Principal Investigator: William A. Fonzi
Agency: National Institutes of Health
Type: R01 (GM47727) Period: April 1, 1997 to March 31, 2001
The aims of this project were to define the functions of the pH-regulated genes PHRl and PHR2 and the mechanism of their pH-dependent regulation. The long term objective is to understand how dimorphism, a potential virulence attribute, is controlled and how this developmental process contributes to virulence.

"Environmental signals and virulence of Candida albicans"
Principal Investigator: William A. Fonzi
Agency: Burroughs Welcome Fund
Type: Scholar Award Period: July 1, 1997 to June 30, 2003
The aims of this project are to develop a method of isolating regulatory mutants using URA3 as a selectable reporter gene. The long term objective is to define the way in which environmental signals are integrated to control dimorphism.

"Niche-specific pathobiology of Candida albicans"
Principal Investigator: William A. Fonzi
Agency: National Institutes of Health
Type: RO1 (AI46249) Period: August 1, 1999 to July 31,2003

The specific aims of this project are to define the function of the cell surface protein encoded by HWPI, to delineate the promoter elements controlling its developmental expression and to examine the relevance of these elements to expression during infection. The long-term objective is to understand the control of gene expression during infection.

"New approaches to target-specific antifungal agents"
Principal Investigator: Ronald L. Cihlar
Co-Investigator: William A. Fonzi
Agency: National Institutes of Health
Type: R01 (CA88456-01) Period: March 24, 2000 to June 30, 2004
The specific aims of this project are to examine the potential of various proteins of Candida albicans as drug targets.

"Candida Albicans Microarrays"
Principal Investigator: Greenspan, John BDS
Co-Investigator: William A. Fonzi
Agency: National Institutes of Health
Type: PO1 (DE07946-14S1) Period: May 1, 2000 to April 30, 2002
The aim of this project is to develop microarray containing all open reading frames of the Candida albicans genome.

B. Selected Peer-Reviewed Publications (in chronological order)

1. Donovan, M., J. J. Schmuke, W. A. Fonzi, S. L. Bonar, K. Gheesling-Mullis, *G.* S. Jacob, V. J. Davisson, and S. B. Dotson, 2000. Virulence of an ADE2 deficient Candida albicans strain in an immune-suppressed murine model of systemic candidiasis. Infect. Immun. In press.
2. Mouyna, I.,T. Fontaine, M. Vai, M. Monod, W. A. Fonzi, M. Diaquin, L. Popolo, R. P. Hartland, and J. P. Latge. 2000. Glycosylphosphatidylinositol-anchored glucanosyltransferases play an active role in the biosynthesis of the fungal cell wall. J. Biol. Chem. 275(20): 14882-14889.
3. Yesland, K., and W. A. Fonzi. 2000. Allele-specific gene targeting in Candida albicans results from heterology between alleles. Microbiology. 146(9):2097-2104.
4. Barkani, A. E., O.Kurzail, W. A. Fonzi, A. M. Ramon, A. Porta, M. Frosch, and F. A. Mühlschlegel. 2000. Dominant active alleles of RIM101/PRR2 bypass the pH restriction on filamentation of Candida albicans. Mol. Cell. Biol. 20(13):4635-4647.
5. Heinz, W. J., O.Kurzai, A. A. Brakhage, W. A. Fonzi, H. C. Korting, M. Frosch, and F. A. Mühlschlegel. 2000. Molecular responses to changes in the environmental pH are conserved between the fungal pathogens Candida dubliniensis and Candida albicans. Int. J. Med. Microbiol. 290(3):231-238.
6. Tsuchimori, N., L. L. Sharkey, W. A. Fonzi, S.W. French, J. E. Edwards, Jr., and S. G. Filler. 2000. Reduced virulence of HWP1-deficient mutants of Candida albicans and their interactions with host cells. Infect. Immun. 68(4):1997-2002.
7. Fonzi, W. A. 1999. PHR1 and PHR2 of Candida albicans encode putative glysocidases required for proper cross-linking of b-1,3- and b-1,6-glucans. J. Bacteriol. 181(22):7070-7079.
8. Ramon, A. M., A. Porta, and W. A. Fonzi. 1999. Effect of environmental pH on morphological development of Candida albicans is mediated via the PacC-related transcription factor encoded by PRR2. J. Bacteriol. 181(24):7524-7530.
9. Sharkey, L. L., M. D. McNemar, S.M. Saporito-Irwin, P. S. Sypherd, W. A. Fonzi. 1999. HWP1 functions in the morphological development of Candida albicans downstream of EFG1, TUP1 and RBF1. J. Bacteriol. 181(17):5273-5279.

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 6162081090000

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: Elitra Pharmaceuticals

*** Start Date:** 07-01-2007 *** End Date:** 06-29-2008 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	John	D.	Trawick		PD/PI	80,000.00	3			20,000.00		20,000.00
2.	Dr.	J	Gordon	Foulkes	PhD	Co-PI	90,000.00	3			22,500.00	0.00	22,500.00
3.	Dr.	William	A	Fonzi	PhD	Investigator	60,000.00	6			30,000.00	0.00	30,000.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** **72,500.00**

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
Total Number Other Personnel						**Total Other Personnel**	
					Total Salary, Wages and Fringe Benefits (A+B)		**72,500.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number:

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 6162081090000

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: Elitra Pharmaceuiticals

*** Start Date:** 07-01-2000 *** End Date:** 06-29-2001 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,500.00
2. Foreign Travel Costs	
Total Travel Cost	**2,500.00**

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

* ORGANIZATIONAL DUNS: 6162081090000

*** Budget Type:** ● Project ❍ Subaward/Consortium

Enter name of Organization: Elitra Pharmaceuticals

*** Start Date:** 07-01-2000 *** End Date:** 06-29-2001 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	14,000.00
2. Publication Costs	
3. Consultant Services	2,350.00
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	**16,350.00**

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**91,350.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Modified Total Direct Costs	10	8,650.00	8,650.00
		Total Indirect Costs	**8,650.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	**100,000.00**

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: 6951-Budget Justification.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification
Personnel:

John Trawick, Principal Investigator (3 calendar months or 25% FTE)
(3 calendar months or 25%FTE)
Dr. Trawick will be responsible for directing the project. This application was originally funded with a modular budget. Subsequent policy changes no longer allow modular budgets for SBIR applications, so we fabricated a detailed budget for this application for the purpose of illustration.

J. Gordon Foulkes (3 calendar months or 25%FTE)
Dr. Foulkes will serve the Co-PI.

William A. Fonzi (6 calendar months or 50%FTE)
Dr. Fonzi will perform many of the studies required for this project.

Travel: $2000
We estimate a total of $2000 will be needed to support travel of the PI to a single scientific meeting during the
course of this project and present an abstract.

Consultants: $2350
A Fee of $2350 is requested to support consultation with Professor William Fonzi, Associate Professor of
Microbiology and Immunology, Georgetown University School of Medicine. Dr. Fonzi has agreed to provide
assistance in the construction of expression vectors and screening in *C. albicans,* and has provided a letter of
support describing his interest in this project.

Equipment costs: $0
No funds have been requested since all the major pieces of equipment needed have already been acquired
and are available for use.

Supplies
Consumables: $14,000.
This budget was based on a historical average of expenditures for laboratory reagents and supplies that were used in the course of similar studies at our facility. Examples of materials included in this category are: enzymes, chemicals, pipet supplies, growth media, Petri dishes, centrifuge tubes, and biochemicals.

Fee
No fee is requested.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		**72,500.00**
Section B, Other Personnel		**0.00**
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		**72,500.00**
Section C, Equipment		
Section D, Travel		**2,500.00**
1. Domestic	2,500.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		**16,350.00**
1. Materials and Supplies	14,000.00	
2. Publication Costs		
3. Consultant Services	2,350.00	
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		**91,350.00**
Section H, Indirect Costs		**8,650.00**
Section I, Total Direct and Indirect Costs (G + H)		**100,000.00**
Section J, Fee		

SBIR/STTR Information

OMB Number: 0925-0001
Expiration Date: 09/30/2007

*** Program Type (select only one)**

● SBIR ❍ STTR

❍ Both *(See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)*

*** SBIR/STTR Type (select only one)**

● Phase I ❍ Phase II

❍ Fast-Track *(See agency-specific instructions to determine whether a particular agency participates in Fast-Track)*

Questions 1-7 must be completed by all SBIR and STTR Applicants:

● Yes ❍ No	* 1. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?
❍ Yes ● No	* 2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies? * If yes, insert the names of the Federal laboratories/agencies:
❍ Yes ● No	* 3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov
● Yes ❍ No	* 4. Will all research and development on the project be performed in its entirety in the United States? If no, provide an explanation in an attached file. * Explanation:
❍ Yes ● No	* 5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work? * If yes, insert the names of the other Federal agencies:
● Yes ❍ No	* 6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?
	* 7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions. * Attach File:

SBIR/STTR Information

OMB Number: 0925-0001
Expiration Date: 09/30/2007

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

○ Yes ● No	* 8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment. * Attach File:
● Yes ○ No	* 9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

○ Yes ○ No	* 10. Please indicate whether the answer to BOTH of the following questions is TRUE: (1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND (2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?
○ Yes ○ No	* 11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?

Attachments

NonDomesticPerformanceExplanation

File Name **Mime Type**

CommercializationPlan

File Name **Mime Type**

SBIR

File Name **Mime Type**

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Project Director / Principal Investigator (PD/PI)

Prefix:
* First Name: John
Middle Name:
* Last Name: Trawick
Suffix:

* New Investigator? ❍No ●Yes

Degrees:

2. Human Subjects

Clinical Trial? ●No ❍Yes

* Agency-Defined Phase III Clinical Trial? ❍No ❍Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: Harry
Middle Name:
* Last Name: Hixson
Suffix:

* Phone Number: 858-410-3030
Fax Number: 858-410-3040
Email: hhixson@elitra.com

* Title: President and CEO

* Street1: 3510 Dunhill St
Street2:
* City: San Diego
County:
* State: CA
* Zip Code: 92121
* Country: USA

Tracking Number:

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Project Director / Principal Investigator (PD/PI)

Prefix:
* First Name: John
Middle Name:
* Last Name: Trawick
Suffix:

* New Investigator? ❍No ●Yes

Degrees:

2. Human Subjects

Clinical Trial? ●No ❍Yes

* Agency-Defined Phase III Clinical Trial? ❍No ❍Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: Harry
Middle Name:
* Last Name: Hixson
Suffix:

* Phone Number: 858-410-3030
Fax Number: 858-410-3040
Email: hhixson@elitra.com

* Title: President and CEO

* Street1: 3510 Dunhill St
Street2:
* City: San Diego
County:
* State: CA
* Zip Code: 92121
* Country: USA

Tracking Number:

OMB Number: 0925-0001
Expiration Date: 9/30/2007

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page and PHS398 Checklist. The responses provided on these pages, regarding the type of application being submitted, are repeated for your reference, as you attach the appropriate sections of the research plan.

*Type of Application:

● New ❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	6185-PHS398RP02SpecificAimsv00.pdf
3. Background and Significance	9710-PHS398RP03Backgroundv00.pdf
4. Preliminary Studies / Progress Report	2494-PHS398RP04PreliminaryStuidesv00.pdf
5. Research Design and Methods	5736-PHS398RP05ResearchDesignMethodsv00.pdf

Human Subjects Sections

Attachments 6-10 apply only when you have answered "yes" to the question "are human subjects involved" on the R&R Other Project Information Form. In this case, attachments 6-10 may be required, and you are encouraged to consult the Application guide instructions and/or the specific Funding Opportunity Announcement to determine which sections must be submitted with this application.

6. Protection of Human Subjects	
7. Inclusion of Women and Minorities	
8. Targeted/Planned Enrollment Table	
9. Inclusion of Children	
10. Data and Safety Monitoring Plan	

Other Research Plan Sections

11. Vertebrate Animals	
12. Consortium/Contractual Arrangements	
13. Letters of Support	1564-Letter of support.pdf
14. Resource Sharing Plan(s)	560-PHS398RP14ResourceSharing.pdf

15. Appendix

Tracking Number:

Attachments

IntroductionToApplication_attDataGroup0

File Name	Mime Type

SpecificAims_attDataGroup0

File Name	Mime Type
6185-PHS398RP02SpecificAimsv00.pdf	application/pdf

BackgroundSignificance_attDataGroup0

File Name	Mime Type
9710-PHS398RP03Backgroundv00.pdf	application/pdf

ProgressReport_attDataGroup0

File Name	Mime Type
2494-PHS398RP04PreliminaryStuidesv00.pdf	application/pdf

ResearchDesignMethods_attDataGroup0

File Name	Mime Type
5736-PHS398RP05ResearchDesignMethodsv00.pdf	application/pdf

ProtectionOfHumanSubjects_attDataGroup0

File Name	Mime Type

InclusionOfWomenAndMinorities_attDataGroup0

File Name	Mime Type

TargetedPlannedEnrollmentTable_attDataGroup0

File Name	Mime Type

InclusionOfChildren_attDataGroup0

File Name	Mime Type

DataAndSafetyMonitoringPlan_attDataGroup0

File Name	Mime Type

VertebrateAnimals_attDataGroup0

File Name	Mime Type

ConsortiumContractualArrangements_attDataGroup0

File Name	Mime Type

LettersOfSupport_attDataGroup0

File Name	Mime Type
1564-Letter of support.pdf	application/pdf

ResourceSharingPlans_attDataGroup0

File Name	Mime Type
560-PHS398RP14ResourceSharing.pdf	application/pdf

Appendix

File Name	Mime Type

Specific Aims

The yearly world market for antimicrobial drugs is over $22 billion, making this the third largest pharmaceutical market. An important and growing component of this market is in the antifungal area. Fungal pathogens such as *Candida albicans*, *Aspergillus fumigatus*, and *Cryptococcus neoformans* cause increasingly problematic diseases in healthy and immunocompromised hosts.

A critical problem in combating fungal infections is that many of the existing antifungal drugs target eukaryotic processes common to both the fungi and mammals. For example, of the several different antifungal drug classes, three target sterol synthesis or directly target plasma membrane sterols (32). The potential and reality of drug toxicity are readily apparent because these drug targets are homologous between fungi and mammals. Picking new targets can be complicated by the many other similarities among all eukaryotes at the levels of cell biology and biochemistry. These facts point to a need to identify and utilize new fungal targets that are absent in humans for the development of more effective and safer antifungal agents.

The most important human fungal pathogen is *Candida albicans*. Capable of switching between yeast and mycelial forms, *C. albicans* causes both topical and systemic infections in humans. Though *C. albicans* is closely related to the well understood model organism, *Saccharomyces cerevisiae*, there are many differences in biology between the two, including the pathogenicity of *C. albicans,* that point to a need for identifying targets and processes within *C. albicans* (26, 31, 32, 40).

One tool that has proven powerful in identifying critical targets is screening for dominant negative phenotypes. Dominant negative screening for genes and phenotypes associated with essentiality will be used to obtain new antifungal targets in *C. albicans*. This fungus has been difficult to study experimentally because of its asexual diploid nature and variant genetic code. The phase I goals are:

1. Construction and optimization of a *C. albicans* site-specific integrating expression vector.

A suitable vector has already been constructed and subjected to preliminary testing. Phase I work will be initiated by cloning the *C. albicans* MET3 promoter into this vector and comparing the induction and repression in *C. albicans* of MET3 promoter or MAL2 (already constructed) promoter driven variations of this vector using a reporter gene. Further optimization and testing of alternative promoters will extend and complete this aim. The vector is a site-specific integrating *C. albicans* expression vector allowing exchange of alternative promoter cassettes. This is to construct the best vector possible for expression library screening and to test this with known dominant lethal genes.

2. This vector will be utilized for production and identification of dominant negative mutants with cDNA libraries; the phenotype to be tested is growth/viability. Libraries will be constructed, mutated, and used in large high-throughput screens to identify *C. albicans* genes that are potential antifungal targets because of their dominant negative phenotype. During this phase, all of the cDNA libraries will be screened to saturation and also subjected to chemical, PCR-based, and deletion mutagenesis and screened to saturation using the high-throughput capabilities at Elitra. As targets are identified, they will be evaluated in terms of their presence in other fungi and in mammals.

3. The ultimate goal in phase II will be to take the new *C. albicans* targets that have been found during phase I, prioritize these based on essentiality in this pathogenic fungus and not in mammals and employ the targets in the cell based assays developed at Elitra. Hits will then be validated and developed into leads for new antifungal drugs.

Background and Significance

Candida albicans is the single most important fungal pathogen in humans (31). In particular, *C. albicans* causes oral and systemic candidiasis in immunocompromised patients and vulvovaginal candiadiasis (VVC) in women. Candidiasis is an extremely important problem in HIVinfected patients, 84 % of who exhibited oropharyngeal colonization by *Candida* spp. in a 1994 study (45). VVC is extremely widespread and a significant medical problem. According to the CDC, some 75 % of women in the USA will have at least one episode of VVC in their lives, 40 % will have two, and a smaller number (~5 %) will have the recurrent form (45). Taken together, this information demonstrates the significant medical and economic importance of *C. albicans* pathogenesis.

There is an increasing need for safer and more effective antifungal agents. Some of the more effective antifungal agents, amphotericin B and the azoles (e.g. fluconazole, itraconazole) have toxicity problems because their cellular targets have homologues in mammalian cells. The azoles inhibit lanosterol 14 α-demethylase, a cytochrome P450 enzyme critical for sterol synthesis in fungi and mammals; the azoles are also effective inhibitors of many cytochrome P450 reactions and because of this are useful tools in mammalian cell biology (27). Amphotericin B targets plasma membrane sterols and is nephrotoxic (32). Additionally, *C. albicans* strains resistant to the azoles have been on the increase in recent years (32).

C. albicans exhibits a complex life cycle dependent upon in vitro and in vivo growth conditions. Normally, *C. albicans* grows as a yeast cell or blastospore at 30° C and with glucose as a carbon source. However, when cultured in the presence of serum, with carbon sources such as N acetyl-glucosamine, at elevated (37 °C) temperatures, or at altered (higher) pH, *C. albicans* switches to a predominantly hyphal form (40). The transitions between the yeast and hyphal forms appear to be essential for virulence. Non-hyphal *C. albicans* strains are avirulent (15) as are obligately hyphal *C. albicans.*

Despite the importance of *C. albicans* to human disease, work on this organism has often been hindered due to its asexual diploid nature and variant CTG codon (in *C. albicans* CTG encodes serine instead of leucine; 28,46). The more facile organism for molecular biology, *S. cerevisiae,* is able to transition between haploid and diploid forms, and is suited to mutagenic analysis of gene function using knock-outs. Though fast and reliable gene disruption methods have been described for *C. albicans* (17,36,60) the lack of sexual cycle along with some manner of inducing meiosis and sporulation means that essentiality of a gene must usually be inferred from negative results. Much work has focused on using *Saccharomyces cerevisiae* as a surrogate model for *C. albicans* genetics and biology, however the many significant genetic, developmental, and pathogenic differences between the two organisms show that much more can be learned by developing methods to study *C. albicans* directly.

There is a critical need to identify new and better targets in *C. albicans* and other pathogenic fungi that can be exploited for antifungal drug development. It is important to perform research directly in *C. albicans* to understand and exploit the unique characteristics of this organism. *C. albicans* is ideally suited to the dominant negative approach because of its diploid nature, complex development, and variant genetic code (20, 33). Dominant genetic methods work because some gene products, often those involved in important regulatory processes, will become trans-dominant inhibitors when the gene is mutated or overexpressed. This methodology is attractive for *C. albicans* because it can be applied to diploid organisms or organisms that lack means for conventional approaches such as targeted gene disruption. In theory, gene products with multiple sites, such as catalytic and regulatory domains that interact with other polypeptide or nucleic acids are potentially susceptible to trans-dominant analysis (20, 49,51). This approach has proven very valuable in a number of systems without conventional genetics (33,38,51), such as mammalian somatic cel culture, and has even proven useful in the classical genetic model, *S. cerevisiae* (42).

In bakers' yeast, *S. cerevisiae*, results of several large-scale screens for dominant negative mutants have been published (30, 43), and have identified genes involved in growth (1, 42), mating type regulation (58), and other processes. Moreover, *S. cerevisiae* has been successfully used as a surrogate background for analysis of *C. albicans* libraries by a dominant negative approach (58), one indication of the similarity of biological functions between the two organisms. Surprisingly, this screen identified a number of dominant genes that interfere with *S. cerevisiae* mating type control of the cell cycle (58). Though lacking a sexual cycle, *C. albicans* does have homologues of the *S. cerevisiae* mating-type genes (21) as well as homologues for the regulators of these genes (13) though the *C. albicans* functions encoded by these genes may vary considerably from their *S. cerevisiae* equivalents.

Dominant negative gene analysis works in *C. albicans*. Both a directed dominant negative (7) and identification of a filamentation-causing dominant gene from a library screen (6) have been reported. Recently, a mutant allele of the Ca-SEC4 gene was overexpressed in *C. albicans* (33). The targeted gene, SEC4, is a Ras-like GTPase that appears to be essential in *C. albicans* and was mutated to mimic a well-characterized trans-dominant mutation in mammalian Ras. The dominant negative allele of SEC4 was successfully used to demonstrate the functional role of SEC4 in cell growth and protein secretion (33).

In *C. albicans*, it is probable that overexpression of some genes can lead to a dominant phenotype (24,33). Some genes in *C. albicans* appear to be uniquely sensitive to minor copy number alterations (22, 39). Regulation of the sorbose utilization gene, SOU1, appears to be through a regulated shift in copy number of *C. albicans* chromosome 5, since monosomic strains assimilated sorbose while non-assimilating disomic strains did not (22). Selection for fluconazole resistant strains of *C. albicans* also resulted in chromosomal copy number shifts (39). Results such as these suggest that there is a distinct possibility that many *C. albicans* genes are regulated through dosage effects and would likely be susceptible to dominant screens involving overexpression.

To identify genes regulating filamentous growth in *C. albicans*, a library was introduced using the REMI method (6). A putative transcription factor with a zinc-finger domain was isolated. Taken together, the two very recent reports (6) of dominant gene isolation in *C. albicans* demonstrate very dramatically the possibilities inherent in working directly *in C. albicans*. Therefore, it would seem likely that larger-scale analysis are possible with suitable vectors and promoters. The goals of this project are to optimize a *C. albicans* expression vector and to exploit the unique characteristics of this vector in dominant negative phenotype screening of *C. albicans* genes involved in growth control and viability of the organism.

The new genes identified in this research will be included in Elitra's unique relational database of both targets and drug screens for all the major gene/protein targets across multiple pathogens. This database will markedly enhance the ability of Elitra and its corporate partners to make informed decisions on which novel targets to pursue. Drug screens will be developed for these novel targets and used to identify new antifungals with high-throughput screening of chemical libraries. Elitra has assembled a team of scientists with extensive experience in developing drug screens for a wide range of targets. Elitra's current library is in excess of 130,000 compounds and can screen at rates in excess of 20,000 compounds per day.

Preliminary Studies

Relevant Experience

The principle investigator for the proposed research project will be Dr. John D. Trawick. Dr. J. Gordon Foulkes will assist the project as co-investigator. Dr. William Fonzi will participate as a consultant. Experimental design and execution will be assisted by Mr. Trung Phuong. The qualifications of the investigators are listed below.

Principal Investigator

Dr. Trawick has over 20 years of experience in molecular biology, genetics, and microbiology in yeasts, bacterial, and mammalian systems. This background has given him extensive experience in the control of gene expression and in the construction and use of plasmid vectors. He graduated cum laude from Gustavus Adolphus College, St. Peter, Minnesota with a B. A. in Biological Sciences. After obtaining a M. S. degree in Biological Sciences from Northern Illinois University, Dekalb, Illinois, he enrolled in the Ph.D. program at the University of Minnesota, Mayo Graduate School of Medicine. Research for his doctoral dissertation, “Control of mini-F plasmid DNA replication” was carried out in the laboratory of Dr. Bruce C. Kline at the Mayo Clinic in Rochester, Minnesota. His research focussed on elucidating the transcriptional interactions involved in plasmid copy number control and DNA replication. He received his Ph.D. in Microbiology from the University of Minnesota in 1984. After obtaining his Ph.D. degree, Dr. Trawick worked in the laboratory of Dr. Michael Getz at the Mayo Clinic under a cancer training grant.

In 1985 Dr. Trawick moved to the laboratory of Dr. Robert O. Poyton in the Department of Molecular, Cellular,and Developmental Biology at the University of Colorado, Boulder, Colorado. In Dr. Poyton’s laboratory, he studied the control of cytochrome oxidase gene regulation and nuclear-mitochondrial interactions in the yeast, *Saccharomyces cerevisiae*. As part of this effort he conducted an extensive study of COX6 gene promoter structure and of the transcription factors acting on this promoter.

In 1990, Dr. Trawick became an Assistant Professor (Adjunct) in the Department of Medicine, University ofColorado Health Sciences Center, Denver. At the Health Sciences Center Dr. Trawick studied the regulation of cholesterol metabolism and bile acid synthesis in mammalian cells within the Hepatobiliary Research Center under Dr. F. R. Simon and Dr. R. A. Davis. From 1992 to 1996, he was an Adjunct Assistant Professor in the Biology Department at San Diego State University Foundation also working on cholesterol metabolism in mammals and collaborating with Dr. R. A. Davis. Dr. Trawick is currently still an Adjunct Research Professor in the Department of Biology at San Diego State University, San Diego, California. While working at the Health Sciences Center and at San Diego State University, Dr. Trawick received grants from the University of Colorado, the American Heart Association of Colorado, and the American Heart Association of California.

In 1997, Dr. Trawick was hired by Dr. Judith Zyskind, founder of Elitra Pharmaceuticals, as the first employee of Elitra Pharmaceuticals; Dr. Trawick is currently head of the yeast (*Candida albicans*) gene identification program.

Co-Principal Investigator

Dr. Foulkes has over 20 years of research experience in signal transduction, molecular biology, and drug discovery. He has managed research teams of over 120 scientists and research budgets of $38MM annually. He trained in several of the world's top research laboratories including Professor Sir Philip Cohen (Univ. of Dundee), Professor Raymond Erikson (now at Harvard) and Professor David Baltimore (currently President of California Institute of Technology), before running his own laboratory as a tenured member of the Medical Research Council, U.K. For the last 12 years, he has headed research efforts in 3 biotechnology companies, Oncogene Science, Aurora Biosciences, and now at Elitra Pharmaceuticals. Previous industrial experience with the SBIR program led to multiple successes, including major collaborations in the anti-infective area with both Biochem Pharma and Sankyo, in addition to internal successes in moving programs forward. His experience also includes advanced engineering automation and drug discovery, through to moving drug candidates into Phase 2 clinical trials.

Research Associate

Trung Phuong has extensive practical experience in a wide range of molecular biology techniques and has expertise that is particularly applicable to this research proposal. He gained experience in expression

vector development and optimization while working at Chiron, Inc. Since moving to Elitra Pharmaceuticals in 1998, Mr. Phuong has had extensive experience in vector development and expression library construction and high throughput screening in several different systems. Mr. Phuong has worked with yeast, bacterial systems, and mammalian systems.

Research Designs and Methods

*Development and optimization of a *C. albicans* expression vector

This proposal requires a vector system for introduction of inducible promoters controlling the expression of *C. albicans* DNA fragments that allows efficient and stable genetic transformation of *C. albicans*. For library-scale transformations and screening, standard plasmid vectors have proven unreliable in *C. albicans* due to the lack of stable replicons. Vectors from other yeast species such as *S. cerevisiae* will not work in this organism. Therefore, a site-specific integrating *C. albicans* expression vector was chosen to facilitate the dominant negative analysis to be proposed in this application.

Previous work in this laboratory has focused on the development of just such an integrating vector system. The site-specific integrating vector pEF/SceTARGET constructed by W. Fonzi (personal communication) was chosen as a backbone for this vector. It contains an ISce I meganuclease site that also has been placed within the genome of *C. albicans* strain CYZ-1 at a disrupted chromosomal copy of the Ca-URA3 gene. When this vector is linearized at the ISce I site and transformed, insertion at the described chromosomal locus within the Ca-URA3 gene is favored. (Fig 1). This vector and all of its derivatives are *C. albicans-E. coli* shuttle vectors with pUC19 replication origins and ampicillin resistance markers to facilitate plasmid DNA production, library or clone construction, and analysis of clones.

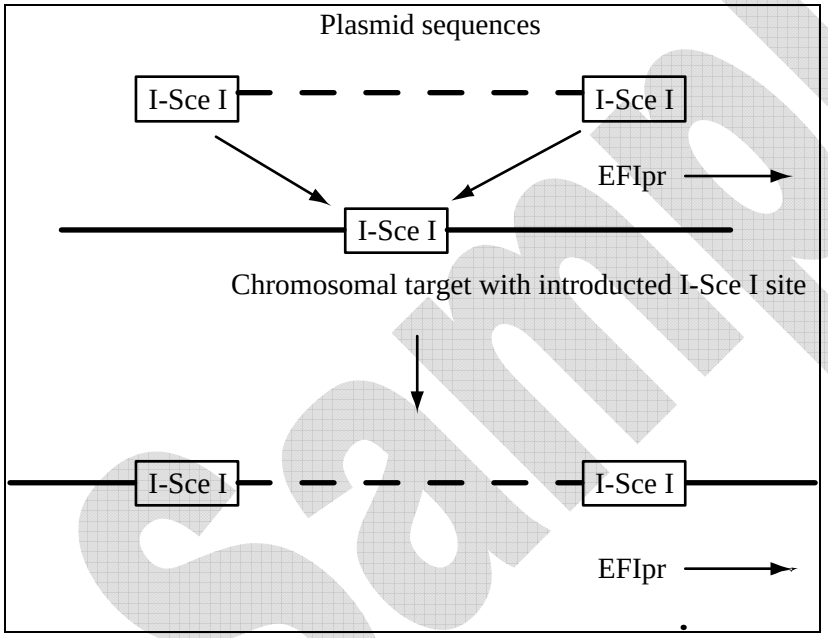

***Figure 1. Site-specific integration using I-Sce I.** The plasmid vector of choice has a single I-Sce I meganuclease site within a region of several hundred base pairs of homology to the chromosomal target (open boxes). To enhance the recombination frequency, a strong, constitutive promoter, Ca-EF1pr (translation elongation factor 1 gene) is oriented towards the I-Sce I site. The plasmid is linearized with I-Sce I and transformed into a host with an identical target region, i.e. the several hundred base pairs of sequence identity, the I-Sce I site, and the promoter. The final integrant should be in a single copy and the Ca-EF1 promoter oriented outwards from the integrated plasmid sequences. The *C. albicans* vector, pEF/SceTARGET has this configuration with the complete Ca-URA3 gene for selection of uridine prototrophy in *C. albicans* and an *E. coli* shuttle vector for propagation in bacteria.

Several episomal plasmids for use in *C. albicans* have been constructed and can be useful when a single gene is to be expressed in *C. albicans* (8, 25, 41) but use of these in expression library-based methods is difficult because of general plasmid instability. Therefore, a site-specific integrating *C. albicans* expression vector is required for ectopic expression of dominant-negative clones in this organism. To construct the needed vector, several components have been assembled and tested (Figures 1and 2 and Tables 1 and 2, below). This *C. albicans* expression vector can efficiently and reliably integrate into a neutral site (Table 1), show highly regulated expression controlled by a *C. albicans* promoter, and will facilitate retrieval of sequences or of the whole vector for characterization or subsequent experiments. When 0.5 µg of

pEF/SceTARGET DNA was transformed into the correct host, CYZ1, around 10^3 transformants per µg resulted, ten-fold better than the number of transformants into the non-specific integrating host, CAI4 (16) (Table 1). This result shows that a site-specific integrating vector can work much more efficiently in *C. albicans* than a randomly integrating vector. This improvement in overall transformation efficiency is necessary for library-scale expression projects in this organism. Though transformation efficiencies for *C. albicans* are still well below levels achievable with *S. cerevisiae*, 10_3 transformants per µg is sufficient for library transformation and even more efficient methods are becoming available (14). Other methods to increase site-specificity of integration, such as restriction enzyme mediated integration (REMI), have also been employed in *C. albicans* for the same purpose (7).

Optimization of the components to the overall technology will likely be required to insure efficient transformation and characterization of *C. albicans* recombinant clones. Preliminary tests and screens using the proposed technology indicate that we will be able to obtain large numbers of *C. albicans* transformants and to screen these for a set of desired phenotypes.

Transformation of *C. albicans* with pEF/SceTARGET

***C. albicans* strain**	**Colonies on SD –ura plates**
CYZ-1	**402, 408**
CAI4	**29, 33**

***TABLE 1.** *C. albicans* strains CYZ-1 and CAI4 are both uridine auxotrophs because both copies of the native URA3 gene have been disrupted (17); in CYZ-1 one of the deleted alleles of the URA3 gene has been replaced with phage λ DNA into which a I-Sce I meganuclease (5'- TAGGGATAA/CAGGGTAAT-3') site has been inserted. No I-Sce I sites are present in the deleted URA3 alleles of CAI4 (17). Into cells of each, 0.5 µg of pEF/SceTARGET were transformed by the Candida-lithium acetate/PEG one-step transformation protocol (10). Transformant colonies were selected for growth on synthetic dextrose drop-out medium lacking uridine (52). Results are from duplicate transformations.

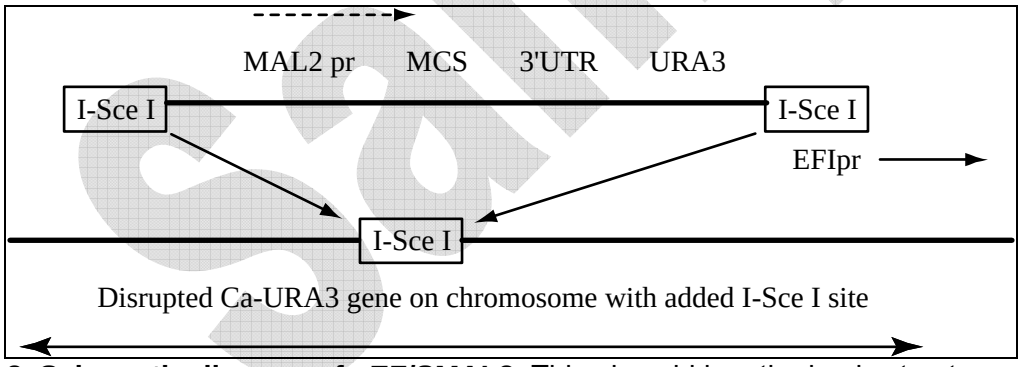

Figure 2. Schematic diagram of pEF/SMAL2. This plasmid has the basic structure and is used as shown in Figure 1. The plasmid features the Ca-MAL2 promoter region (500 bp) or the MET3 promoter region (9). A multiple cloning site, and a yeast 3'-UTR region in addition to the URA3 selectable marker for *C. albicans* and the *E. coli* shuttle sequences for bacterial propagation are also present(but not depicted).

Promoter choice and requirements.

The best possible properties of a promoter for an expression vector are repressibility to zero background and strong inducibility, with induction and repression mediated by factors which do not otherwise affect growth or differentiation. Due to the variant genetic code, it hasn't been possible to adapt promoters requiring transcription factors derived from other species, so *C. albicans* molecular genetics has lagged. Very recently, the MET3 promoter from *C. albicans* has been cloned and demonstrated to by nearly completely repressed when cells are grown in the presence of methionine or cysteine (9). When both of these amino acids were present at 0.5 mM the MET3 promoter was fully repressed (9). Furthermore, when these amino acids were removed from the culture medium, an induction of 85 fold occurred (9). Switching the MET3 promoter on or off by removal or addition of methionine or cysteine does not affect *C. albicans* growth or

morphology and control can be accomplished in host strains prototrophic for these amino acids. Therefore, the MET3 promoter sequences will be isolated and cloned as described (9) into the pEF/SceTARGET vector. The minimal length of the MET3 promoter appears to be 1362 bp (9). The MET3 promoter fragment will replace a cassette that contains the 500 bp Ca-MAL2 promoter to create pEF/SMET3, in addition to pEF/SMAL2. This will result in two different expression vectors, one with the MET3 promoter and the other with the maltase promoter (7, 12, 18). The MAL2 promoter controls the maltase gene and is repressed when glucose is added to the growth medium, and induced when maltose is added. The resulting plasmid also contains a multiple cloning site, and the 3' UTR and transcriptional termination region of the Sc-CYC1 gene. The latter will insure proper termination of transcription, cleavage and polyadenylation. Transcriptional terminators and cleavage and polyadenylation regions are generally interchangeable among the yeasts and fungi (19, 23), therefore it is likely that these *S. cerevisiae*-derived transcription termination elements will work in *C. albicans*. In the unlikely event that proper termination and mRNA processing does not occur with this region, a substitute from an endogenous *Candida albicans* gene such as the maltase gene will be cloned into the vector. (Fig 2). While the MET3 promoter driven expression vector will be the first choice and the MAL2 promoter vector, the second, a few alternative inducible promoters are available for *C. albicans*. These include HWP1, repressed by glucose and induced by serum and 37˚ C incubation (50, 56), PHR1, which is subject to pH-titratable control (37, 47), and the GAL1 promoter, though apparently not regulated as tightly as in *S. cerevisiae,* it is induced strongly by galactose (32,33).

The repressed state of a promoter on an expression vector should be complete to insure that leakage from the promoter does not cause loss of some clones from the library. However, most eukaryotic promoters do retain some small basal expression (55). This is why the MET3 promoter looks so attractive; no measurable growth occurred in *C. albicans* cells deleted for the native alleles of URA3 when a copy of URA3 controlled by the MET3 promoter was fully repressed (9). This repression was stronger than that seen for other regulated *C. albicans* promoters (9, 12). Prior to the characterization of the MET3 promoter, three *C. albicans* promoters were tested and compared (Table 2). Two of these (MAL2 and HWP1) were strongly repressed by a LacZ reporter assay and were strongly induced.

LacZ Assays in *C. albicans* CYZ-1

Promoter	Glucose (repressing)	Maltose/Sucrose	Serum
MAL	<0.3	1.73—4.2	n.d.
ACT	14.6	27	23
HWP	<0.1	n.d.	6-17

***TABLE 2.** LacZ fusions to maltase (Mal), actin (ACT), and HWP1 promoters were transformed into *C. albicans* CYZ-1 and URA+ transformants selected. Cultures were grown and assayed as suggested in Uhl and Johnson, 1999 and by Uhl (personal communication). The units are in β-galactosidase units from the ONPG assay (34). The variation is due to multiple experiments with variables in growth time and state.

Expression must be sufficient to produce enough of the gene product to interfere with normal activity of that enzyme. This need not be equivalent to the expression level of the native gene if the effect is dominant. Hypothetically, a single dominant negative polypeptide normally part of a homotetramer could, in principle, poison the active tetrameric complex. However, for overexpression-based gene identification, overall expression should be higher than total endogenous expression. The strongest described *C. albicans* promoters are probably those for genes such as actin (35), which is largely constitutive. The recently described MET3 promoter also appears to be very strongly induced (9), the 85 fold induction observed approaches the apparent maximum seen in microarray assays of the related organism, *S. cerevisiae* (48, 16).

To test whether or not our promoter/vector system is sufficient for this kind of gene identification, the CA-actin and/or tubulin genes will be cloned into the vector/promoter as outlined for libraries above and transformed into *C. albicans* CYZ-1. Overexpression of either actin or tubulin is sufficient to inhibit growth in *S. cerevisiae* (30). If overexpression of the *C. albicans* actin gene is sufficient to cause a growth defect, this will be observed under the inducing but not repressing conditions. Failure to overexpress actin or tubulin sufficiently to block growth or failure to overexpress TUP1 sufficiently to block filamentation will require use of an alternative promoter or mutagenesis of the existing promoter to increase expression.

***Reporter gene for C. albicans**

The *Streptococcus thermophilus* lacZ gene (Material Transfer Agreement in place) functions as a reporter of gene expression in *C. albicans*. We have used this reporter to test the Ca-MAL2, Ca-HWP1 promoters, as well as alternative promoters such as the Ca-ACT1 promoter. To initiate the phase I study, the MET3 promoter will be cloned into pEF/SceTARGET as described (9). Then, the *S. thermophilus* lacZ gene will be used as a reporter to compare expression to other available *C. albicans* promoters. Preliminary results indicate that the MAL2 and HWP1 promoters are highly controllable, with strong repression and induction (up to several β-galactosidase units for single copy integrants) (Table 2). Judging from the published report (9), the MET3 promoter is expected to be completely repressed and more strongly induced. The site-specific integrating vector, pEF/SMAL2 has been constructed but a variant with the MET3 promoter needs to be constructed.

Cloning of lacZ reporter into the site-specific integrating vector.

The appropriate restriction fragment of the *Streptococcus thermophilus* lacZ gene will be obtained by restriction digestion or PCR amplification and cloned into the multiple cloning region of pEF/SMET3 or pEF/SMAL2. After the structure and sequence of the recombinant plasmid has been confirmed, it will then be transformed into *C. albicans* strain CYZ-1 and transformants selected on minimal medium minus uridine (17, 52, a.k.a. SD –ura). The location and copy number of a small random collection of transformants will be examined by genomic DNA purification followed by PCR amplification. These recombinant *C. albicans* strains will be assayed for β-galactosidase activity when grown in +/- methionine-cysteine synthetic dextrose medium for repression or induction of MET3. The MAL2 promoter will be assayed in repressing (glucose) and inducing (maltose) medium over a suitable time course. Alternatives to this strategy are: use of random site integrating vectors that allow efficient regulated expression but do carry the risk of causing mutations upon integration; alternative promoters: PHR1(37), induced by pH, and ACT1 (35) which is largely constitutive; construction of different site-specific integrating vectors that target another non-essential *C. albicans* allele.

Gene retrieval methodologies.

Colony PCR from yeast colonies will be used to obtain the sequences of the cDNA giving the desired phenotype(s). Several efficient colony PCR methods from yeast, as well as bacterial colonies, are available (29, 53) and are standard methods in use at Elitra. Rescue of integrated plasmids by restriction digestion and ligation (44) will be employed to obtain the whole recombinant plasmid. The I-Sce I site within the vector and targeting site in the *C. albicans* genome will be exploited in this protocol. Genomic DNA from *C. albicans* that contains the integrated plasmid will be purified by standard means and completely digested with meganuclease I-Sce I. Then, the digested DNA will be ligated with T4 DNA ligase and electroporated into electrocompetent *E. coli* TOP10 cells (Invitrogen, Carlsbad, CA) with an efficiency of 10_9 transformants per microgram. Rescued plasmids will be selected for ampicillin resistance and confirmed by restriction endonuclease digestion and DNA sequence analysis.

Identification of dominant negative genes in C. albicans.

The vector/promoter system described and tested above, will be used to identify genes important to *C. albicans* growth and viability using a dominant negative approach. To identify dominant negative genes in *C. albicans*, full-length cDNA libraries will be constructed. These will be both unmutagenized and mutagenized (see below). A directed orientation cDNA library will be made by enriching poly A-containing RNA from total RNA using oligo dT cellulose according to standard methods (3). The first strand of cDNA will be made from purified poly A RNA by reverse transcriptase; second strand synthesis will be via DNA polymerase I. Commercial kits from supplies such as Life Technologies (Gaithersberg, MD) will be used to synthesize cDNA. Size selection of double-stranded cDNA will be performed to insure that predominantly full-length cDNA clones are synthesized. The cDNA library will be directionally cloned into pEF/SMET3 or pEF/SMAL2 by using primer/adapters for cDNA synthesis with specific restriction endonuclease sites to confer directionality (e.g. the 3', oligo-dT primer/adapter may have a NotI site and the 5' primer adapter a SalI site). To ascertain the quality of the cDNA library, random clones will be isolated and the DNA sequence of the inserts determined. A high quality library will be judged to contain fewer than 5 % vector lacking inserted clones, and the cDNA clones will be largely or entirely full-length and containing coding regions with total numbers of unique recombinant plasmids sufficient to saturate the genome. Identification of genes will be through BLAST searches of the Candida (http://sequence-www.stanford.edu/group/candida/ and

http://alces.med.umn.edu/Candida.html), Saccharomyces (http://genome-ww.stanford.edu/Saccharomyces/), and NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) public databases.

An advantage of cDNA libraries is that only coding regions, and, if size-selected, predominantly intact genes are cloned. This will force expression of clones containing genes expressed from their native start codons.

The cloned library DNA will be linearized within the vector using I-Sce I and then transformed into *C. albicans* strain CYZ-1 and integrants selected on minus-uridine medium. The extreme rarity of I-Sce I recognition sites means that it is unlikely that any cDNA inserts will contain a recognition sequence for the meganuclease. Transformant clones will be plated onto repressing medium (e.g. containing methionine and cysteine if the MET3 promoter is used). A high-throughput replica plating method will be used to identify dominant negative clones; i.e. clones that do not grow when induced. Colonies from a plated library will be picked and transferred to liquid repressing medium in 384 well plates with a robotic colony picker (GeneMachines, Inc, San Carlos, CA). This colony picking and inoculating device can aseptically identify and pick colonies from transformant plates at the rate of 2,000 colonies per hour; colonies are transferred to recipient plates containing liquid growth medium. After a growth period, these clones will then be robotically replica plated (BioGrid replica gridding device from BioRobotics, Ltd., Cambridge, U.K.) onto both repressing (+0.5 mM methionine/cysteine) and inducing (0 mM methionine/cysteine) plates. Robotic colony picking and replica plating enable the screening of 10,000 to 50,000 colonies per day at Elitra.

In screens for growth inhibitory genes, dominant negative clones will be those that grow on repressing medium and that fail to grow on inducing medium. Growth inhibition such as this may be very strong with a complete or nearly complete inhibition of growth. Alternatively, weaker growth inhibition might also be observed. This type of phenotype will be apparent on the robotically gridded plates but is subject to gridding artifacts. Plating serial dilutions of all putative growth inhibitors from the initial scoring of sensitives will be done to confirm the phenotype and score the relative strength of the phenotype.

As with all dominant gene technologies, the principle is to identify an essential process or interaction. Overexpressing a component of an essential multicomponent pathway may disrupt the whole pathway (20, 59). Several assayable phenotypes including growth (viability) and filamentation are suitable for screening by overexpression. For instance, the CA-TUP1 gene is a repressor of filamentation in *C. albicans* and is a factor in the control of this critical morphogenic pathway (5). One would predict that overexpression of TUP1 might block filamentation even under conditions known to induce TUP1-dependent filamentation such as serum and incubation at 37˚ C (5). In *S. cerevisiae*, genes that block growth (1, 30) or cause various phenotypic changes (43, 58) have been discovered by this method. Lethal overexpression could also include events that interfere with viability by unbalancing concentration sensitive pathways such as actin and tubulin assembly

The phenotype of clones sensitive to induction will be retested by regridding to confirm the identification of potential positive clones. Certain classes of false positive clones can be expected. *S. cerevisiae* transformations sometimes yield high rates of petite mutations. Such artifacts can be checked by comparing specific phenotypes, monitoring induction of the phenotype or by selecting for plasmid loss on counter-selective medium (5-fluourorotic acid containing plates will counter select URA3 genes in *C. albicans* as well as *S. cerevisiae*). Another type of artifact could be the illegitimate insertion of a plasmid into a gene, causing a mutation in cis. True sensitives will then be identified by PCR, inverse PCR, or plasmid rescue cloning, all followed by DNA sequencing. Artifactual production of mitochondrial petites may be much less likely with *C. albicans* than with *S. cerevisiae* because of the paucity of reports of petite and, therefore, respiration-deficient *C. albicans* strains compared to *S. cerevisiae* (2). All types of artifacts can be screened for by retransforming the identified clones into the parental *C. albicans* strain and confirming that the phenotype is due to the recombinant plasmid and not to some other factor.

Creation of mutagenized cDNA libraries.

The approach outlined above depends on ectopic expression or overexpression of genes resulting in a dominant negative phenotype. There is considerable precedent for this phenomenon (30, 43). However, some dominant phenotypes require point mutations altering an enzymatic activity (33) while deletion mutations may favor other dominant phenotypes (20, 49, 51). To this end, cDNA will be made from *C. albicans* mRNA as described above. Then the DNA will be subjected to an in vitro mutagen. One such mutagen is hydroxylamine that hydroxylates cytosine residues and leads to a transition mutation after replication (54). The mutated DNA will be purified from the mutagen and then cloned into the vector, making a mutant genomic DNA library. Alternatively, mutagenic PCR amplification of the library may also provide a

means of obtaining the desired mutants. This methodology greatly decreases the likelihood of multiple factors (e.g. promoter mutation and gene mutation) confounding the analysis. Transformation, selection, screening and gene identification will proceed as described above.

A cDNA library containing deletions will also be constructed. As discussed further above, this approach is more likely to identify membrane or secreted proteins that are toxic when overexpressed. These artifacts will be eliminated postscreening by using bioinformatic methods (GCG Wisconsin package programs) to identify domains of dominant negative clones. Deletion mutations will be constructed by synthesizing cDNA as described above and subjecting the cDNA to restriction enzyme digestion, DNAseI treatment, or mechanical shearing. In all cases the 5' (and therefore amino terminal) end will contain the SalI cloning site attached during second strand cDNA synthesis. Therefore, the library will mainly contain clones that will still initiate translation from their endogenous start codons. To insure proper termination of translation, stop codons in all three reading frames will be introduced 3' of the downstream cloning site and upstream of the transcription termination region.

As with any technology, there are limitations to the kinds and numbers of genes that can be identified. Essentiality or cell viability changes due to dominant negative expression may differ from effects seen in knockouts of the same genes. However, dominant negative screens, by their very nature (20, 38, 42, 49, 59), target essential reactions, complexes, or interactions. To determine if a particular gene in *C. albicans* is essential, null alleles of these genes will be created by the *C. albicans* URA-blaster technique (17). The limitation, due to the lack of a *C. albicans* sexual cycle, is that essentiality of a gene is seen as a negative result. Placing the gene under an inducible promoter and disrupting the normal alleles of the gene is the singular way to definitively demonstrate essentiality in *C. albicans* (4, 9). However, dominant negative mutants from mutagenized clones (deletions or point mutations) are more likely to produce a higher fraction of true essential genes. Another qualification to this type of technology is that it will not identify all possible essential genes nor will it identify all steps in a pathway. There is a theoretical requirement that a dominant mutant protein must have at least two domains—one of which will promote protein-protein or protein-nucleic acid interactions (49). However, the *C. albicans* genome (11, 26, 57) contains between 6,000 and 8,000 genes with ~15-17 % likely to be essential; a subset of these essential genes will be identified with the dominant negative approach.

Bioinformatics and target prioritization.

Targets in C. albicans that have been identified by this process will be included in the proprietary database of essential genes that Elitra Pharmaceuticals has been building. Each target will be evaluated against a set of criteria including presence or absence in other eukaryotic pathogens and presence or absence in the human or other mammalian genomes. The most desirable targets for antifungals might, in principle, be those that are found only in pathogens and do not have any human counterparts.

Summary

To identify both essential genes and new virulence targets in the human fungal pathogen, *C. albicans*, a dominant negative gene identification approach will be tested. Some preliminary work on transformation, testing of promoters and reporters, and the construction of a site-specific integrating expression vector has already been accomplished. The focus of this phase I application will be to complete optimization of the vector, demonstrate the feasibility of dominant gene technology in *C. albicans*, construct the first libraries to be screened, and to identify as many targets as the technology will permit. Therefore, cDNA libraries will be screened to saturation, mutated, and those libraries screened to saturation. Multiple libraries will be generated to produce (*i*) intact, full-length cDNA clones for overexpression studies, (*ii*) deletion or partial length cDNA clones for dominant negative screens, and (*iii*) mutated libraries for dominant negative screens. Additionally, cDNA will be produced from mRNA isolated from *C. albicans* cells grown in a variety of growth condition and developmental phases.

Phase II

Phase I of this SBIR proposal will accomplish a number of goals. These include validation of our *C. albicans* expression vector, development of the capability of large scale and high throughput handling of *C. albicans* molecular biology, and identifying dominant negative targets in *C. albicans*. The experimental approaches that I have described will clearly allow us to prove the efficacy of this methodology for *C. albicans* using cDNA libraries. An added benefit is that we will likely identify some potentially useful *C. albicans* targets

and be ready for a much broader application of this methodology during Phase II of the SBIR. Envisioned are the identification of approximately 100 *C. albicans* essential targets during Phase I and Phase II. During Phase II, 3 to 5 of these targets will be validated and screened against ~150,000 compounds.

The new genes identified in this research will be included in Elitra's unique relational database of both targets and drug screens for all the major gene/protein targets across multiple pathogens. Using information gained from both the screens and bioinformatics, targets will be prioritized, drug screens will be developed using either a cell-based assay or a purified target assay to facilitate the screening of chemical libraries to obtain candidates for new antifungal drugs.

RESOURCE SHARING

Sharing plans were not required at the time this application was submitted. The following information was generated for illustrative purposes only.

Data Sharing Plan

Sharing of data generated by this project is an essential part of our proposed activities and will be carried out in several different ways. We would wish to make our results available both to the community of scientists interested in [this disease] and the biology of [its causative agent] to avoid unintentional duplication of research. Conversely, we would welcome collaboration with others who could make use of the vaccine assessment protocols developed in [the project].

Our plan includes the following:

Presentations at national scientific meetings.

From the projects, it is expected that approximately four presentations at national meetings would be appropriate. There is an annual [Disease] Study Group meeting, of which the PI is secretary. This one-day meeting of interested persons presents new information on a variety of topics related to [the disease]. It is expected that the investigators from this [project] will be active participants of this focused group.

Annual lectureship.

A lectureship has brought to the University distinguished scientists and clinicians whose areas of expertise were relevant to those interested in [the disease]. Lecturers have been [list of names]. Visiting lecturers will be scheduled to interact with the investigators of the project as appropriate with their specific areas of expertise which will provide an opportunity for members to present their work to the visitor.

Newsletter.

The [disease interest group] publishes a newsletter which currently has a circulation of [number]. The newsletter's intent is to disseminate new information regarding [the disease]. The activities and discoveries of [the project] will be allocated 20% of the newsletter's coverage.

Web site of the Interest Group.

The [interest group] currently maintains a Web site where information [about the disease] is posted. Summaries of the scientific presentation from the [quarterly project] meetings will be posted on this Web site, written primarily for a general audience. [Link to Web site]

Annual [Disease] Awareness week.

Beginning this fall during the week of [date], the [interest group] will be sponsoring a [Disease] Awareness week. As part of that program, there will be a research poster display with discussions. In future years, [the project investigators] will be active participants in this program.

SAGE Library Data.

[This project] will generate data from several SAGE libraries. It is our explicit intention that these data will be placed in a readily accessible public database. All efforts will be made to rapidly release data through publication of results as quickly as it is possible to analyze the experiments. Data used in publications will be released in a timely manner. SAGE data will be made accessible through a public site that allows querying as has been set up for a similar project. This site can be accessed at [link to Web site].

Model Organism Sharing Plan

See the NIH Model Organism Sharing Policy Web site for sample plans, FAQs, and more.
http://grants.nih.gov/grants/policy/model_organism/index.htm

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December 12, 1999

John D. Trawick, Ph.D.
Senior Research Scientist
Elitra Pharmaceuticals, Inc.
3510 Dunhill Street
San Diego, CA 29121

Dear John,

This letter is to express my willingness to participate in the research project described in your NIH Phase I SBIR proposal, "Dominant Expression Libraries to Obtain New Targets in *Candida albicans*." My contribution will be as a paid consultant to Elitra Pharmaceuticals.

Sincerely,

William A. Fonzi

William A. Fonzi
Associate Professor

3900 Reservoir Road NW Washington, DC 20007-2197

PHS 398 Checklist

OMB Number: 0925-0001
Expiration Date: 9/30/2007

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

● New ❍ Resubmission ❍ Renewal ❍ Continuation ❍ Revision

Federal Identifier: AN2921331

2. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

❑ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ❍ No ❍

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ❍ No ❍

Tracking Number:

OMB Number. 0925-0001
Expiration Date: 9/30/2007

4. Program Income

Is program income anticipated during the periods for which the grant support is requested?

○Yes ●No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. Assurances/Certifications (see instructions)

In agreeing to the assurances/certification section 18 on the SF424 (R&R) form, the authorized organizational representative agrees to comply with the following policies, assurances and/or certifications when applicable. Descriptions of individual assurances/certifications are provided at: http://grants.nih.gov/grants/funding/phs398/PolAssurDef.doc

*Human Subjects; *Research Using Human Embryonic Stem Cells; *Research on Transplantation of Human Fetal Tissue; *Women and Minority Inclusion Policy; *Inclusion of Children Policy; *Vertebrate Animals; *Debarment and Suspension; *Drug- Free Workplace (applicable to new [Type 1] or revised [Type 1] applications only) ; *Lobbying; *Non-Delinquency on Federal Debt; *Research Misconduct; *Civil Rights (Form HHS 441 or HHS 690); *Handicapped Individuals (Form HHS 641 or HHS 690); *Sex Discrimination (Form HHS 639-A or HHS 690); *Age Discrimination (Form HHS 680 or HHS 690); *Recombinant DNA and Human Gene Transfer Research; *Financial Conflict of Interest (except Phase I SBIR/STTR); *Prohibited Research; *Select Agents; *Smoke-Free Workplace; *STTR ONLY: Certification of Research Institution Participation.

If unable to certify compliance, where applicable, provide an explanation and attach below.

Explanation:

Tracking Number:

Attachments

CertificationExplanation_attDataGroup0

File Name	Mime Type